dFMRP and Caprin, translational regulators of synaptic plasticity, control the cell cycle at the Drosophila mid-blastula transition

Ophelia Papoulas1,‡,*, Kathryn F. Monzo1,*,†, Greg T. Cantin2, Cristian Ruse2, John R. Yates, III2, Young Hee Ryu1 and John C. Sisson1

SUMMARY

The molecular mechanisms driving the conserved metazoan developmental shift referred to as the mid-blastula transition (MBT) remain mysterious. Typically, cleavage divisions give way to longer asynchronous cell cycles with the acquisition of a gap phase. In Drosophila, rapid synchronous nuclear divisions must pause at the MBT to allow the formation of a cellular blastoderm through a special form of cytokinesis termed cellularization. Drosophila Fragile X mental retardation protein (dFMRP; FMR1), a transcript-specific translational regulator, is required for cellularization. The role of FMRP has been most extensively studied in the nervous system because the loss of FMRP activity in neurons causes the misexpression of specific mRNAs required for synaptic plasticity, resulting in mental retardation and autism in humans. Here, we show that in the early embryo dFMRP associates specifically with Caprin, another transcript-specific translational regulator implicated in synaptic plasticity, and with elf4G, a key regulator of translational initiation. dFMRP and Caprin collaborate to control the cell cycle at the MBT by directly mediating the normal repression of maternal Cyclin B mRNA and the activation of zygotic frühstart mRNA. These findings identify two new targets of dFMRP regulation and implicate conserved translational regulatory mechanisms in processes as diverse as learning, memory and early embryonic development.

KEY WORDS: Cyclin B, Fragile X syndrome, Frühstart (Z600), Drosophila

INTRODUCTION

The mid-blastula transition (MBT) is defined as the first developmental event that requires zygotic gene activity and represents a critical transition in animal development, but the molecular regulatory mechanisms that control the proper timing of the MBT are only partially understood (reviewed by Tadros and Lipshitz, 2009). Initially, embryos are subdivided through cleavage without cell growth. However, when a species-specific nucleocytoplasmic (N:C) ratio is achieved, the MBT is triggered, a developmental event typically characterized by a dramatic increase in the length and asynchrony of subsequent cleavage division cycles. Preceding the MBT, animal embryos must undergo a controlled degradation of maternal transcripts and activation of the zygotic genes in a precise hand-off of genetic control known as the maternal-to-zygotic transition (MZT).

In Drosophila, both the degradation of maternal transcripts and the wholesale activation of the zygotic genome are largely driven by a timing mechanism and are independent of the N:C ratio, although they can profoundly impact the morphological events of the MBT (Benoit et al., 2009; Lu et al., 2009). For example, loss of function of smaug (smg), a key regulator of maternal mRNA decay, results in the failure of many downstream processes, including activation of the DNA damage checkpoint, cell cycle slowing, cellularization and the transcription of many zygotic genes (Benoit et al., 2009). However, many specific aspects of the Drosophila MBT, including cell formation at nuclear cycle 14 (NC14) and the activation of specific zygotic genes, are believed to be triggered by unknown signals stemming from the N:C ratio (Edgar et al., 1986; Lu et al., 2009). This has been demonstrated in part through analysis of Drosophila maternal haploid (mh) mutants. The haploid embryos derived from mh mothers develop normally until NC14. However, they then undergo an additional nuclear division to achieve the necessary N:C ratio prior to extending interphase and undergoing cellularization (Edgar et al., 1986). The molecular nature of the N:C signal remains elusive, but ultimately impacts Cyclin-dependent kinase 1 (CDK1, also known as CDC2) through multiple mechanisms. These include modulation of Cyclin B (CYCB) levels through rounds of protein synthesis and degradation (Edgar et al., 1994; Huang and Raff, 1999; Raff et al., 2002), activation of the grp (Chk1) and mei-41 (at1) DNA damage checkpoint pathway (Fogarty et al., 1997; Sibon et al., 1997; Sibon et al., 1999; Royou et al., 2008), and precisely timed zygotic transcription of the mitotic cyclin-dependent kinase (M-CDK1) inhibitors frühstart (frs; Z600 – FlyBase) and tribbles (trbl) (Grosshans and Wieschaus, 2000; Mata et al., 2000; Grosshans et al., 2003; Gawlinski et al., 2007).

It is clear that degradation of maternal mRNA and initiation of zygotic transcription contribute to the timing and morphological events of the MBT (Arbeitman et al., 2002; Tadros and Lipshitz, 2005; Pilot et al., 2006; De Renzis et al., 2007; Lu et al., 2009; Tadros and Lipshitz, 2009); however, the translational regulatory mechanisms that modulate rates of protein synthesis during this transition are largely unexplored. We previously found that the transcript-specific translational regulator, dFMRP (FMR1 –
FlyBase), is required for the major morphological event of the MBT, i.e. cellularization (Monzo et al., 2006). In this study, we identify proteins that are associated with dFMRP and demonstrate that dFMRP collaborates with one of these, Caprin, to ensure correct timing of the MBT. dFMRP and Caprin associate with both CycB and frs mRNAs, but function to activate translation of one target while repressing translation of the other to appropriately modulate the cell cycle at the MBT. The identification of Caprin as a partner for dFMRP in this novel context, the MBT, suggests that these proteins might respond together to diverse developmental signals.

MATERIALS AND METHODS

Genetics

Stocks were reared on standard cornmeal molasses media. Oregon-R (wild type), w^{118}, w^{108}, Df(3R)Exel2625, P[X-P-U] Exel2625/TM6B Tb^b, y^w{118} ; P[EPvg2/CG18811]Fv06062; w; Df(3L)Cat ri std eTM6 Ser from the Bloomington Stock Center (University of Missouri, IN, USA), w^{115}, fnr1^{1} TM6C, Tb and Sh from Th. S. Jongens (University of Pennsylvania, Philadelphia, PA, USA), and CyO, P[w^{118} = GAL4-lwi.G2.2, P(UAS-2xEGFP)A1H2.2 (referred to as Cyc-GFP) was from D. Stein (The University of Texas at Austin, TX, USA).

To generate Capr alleles, homozygous y^{w{118}} w^{108} females were crossed to y, w; CyO, y^{1} w^{118} Sh 1/2-3/TM6 males. Genomic DNA from single y, w; p^{excision}/Balancer males was screened by PCR (Gloor et al., 1993) to characterize deletions, using primers 2+ (5'-GACATGTTAGGTTAGTGGG-3'), 3– (5'-ACCTG- GTCAACAACCTTGC-3') and 4– (5'-CGCATGACTCCGATTGG-3'). The corresponding genomic region of four viable deletions (520, 648, 707 and 973 bp) and a precise excision event designated Df(3L)Cat ri std eTM6 Ser were from the Bloomington Stock Center (Bloomington, IN, USA), w^{115}, fnr1^{1} TM6C, Tb and Sh from Th. S. Jongens (University of Pennsylvania, Philadelphia, PA, USA), and CyO, P[w^{118} = GAL4-lwi.G2.2, P(UAS-2xEGFP)A1H2.2 (referred to as Cyc-GFP) was from D. Stein (The University of Texas at Austin, TX, USA).

Biochemistry and molecular biology

Except as noted, procedures were performed according to Sisson et al. (Sisson et al., 2000). 'Protein null' embryos were obtained from Capr^{1}/Df(3L)Cat (Capr) and dfrn^{1}/Df(3R)Exel2625 (dfrn) females. CDC2 phospho-isoforms were resolved on a 20-cm long, 1-mm thick 10.5% PAGE gel at 300V for 8 hours at 4°C prior to transfer to PVDF. 'Protein null' embryos were obtained from Df(3L)Cat ri std eTM6 Ser (Sisson et al., 2000). 'Protein null' embryos were obtained from Df(3L)Cat ri std eTM6 Ser (Sisson et al., 2000). 'Protein null' embryos were obtained from Df(3L)Cat ri std eTM6 Ser (Sisson et al., 2000). 'Protein null' embryos were obtained from Df(3L)Cat ri std eTM6 Ser (Sisson et al., 2000).

Multidimensional protein identification technology (MudPIT)

Protein samples were digested with trypsin as previously described (Link et al., 1999). The protein digest was pressure-loaded onto a fused silica capillary biphasic column containing a 3 cm of 5 μm Aqua C18 material (Phenomenex, Ventura, CA, USA) followed by 2 cm of 5 μm Partisipore strong cation exchanger (Whatman, Clifton, NJ, USA) packed into a 250 μm internal diameter capillary with a 2 μm filtered union (UpChurch Scientific, Oak Harbor, WA, USA). The biphasic column was washed with buffer A (94.9% water, 5% acetonitrile, 0.1% formic acid). After desalting, a 100 μm internal diameter capillary with a 5 μm pulled tip packed with 10 cm 3 μm Aqua C18 material and the entire split-column (biphasic column-filter union-analytical column) were placed inline with a 1100 quaternary HPLC (Agilent, Palo Alto, CA, USA) and analyzed using a modified four-step separation as described previously (Washburn et al., 2001). Step 1 consisted of a 100-minute gradient from 0-100% buffer B (80% acetonitrile/0.1% formic acid). Steps 2-4 were: 3 minutes of 100% buffer A, 2 minutes of X% buffer C (500 mM ammonium acetate/5% acetonitrile/0.1% formic acid), a 10-minute gradient from 0-15% buffer B, and a 97-minute gradient from 15-45% buffer B; the 2-minute buffer C percentages (X) were 20, 40 and 100%, respectively, for the four-step analysis.

Estimates were subjected to a global analysis of a MudPIT (Lindahl et al., 2004) using three biological replicates. All MS/MS spectra were searched against the PDB database of reversed sequences (Peng et al., 2003). All searches were performed using the SEQUEST algorithm (Edgar et al., 1999) against the EBI-IPI database of reversed sequences (Peng et al., 2003). All searches were performed using the SEQUEST algorithm (Edgar et al., 1999) against the EBI-IPI database of reversed sequences (Peng et al., 2003).

Imaging

Live analysis of cellularization was performed as described (Monzo et al., 2006). Time-lapse differential interference contrast (DIC) imaging of cortical nuclear divisions in live embryos was performed using a Zeiss.
A role for dFMRP and Caprin at the MBT

RESULTS
dFMRP associates with Caprin and eIF4G in early Drosophila embryos

To define the mechanism of dFMRP function during the MBT we set out to identify protein binding partners of dFMRP and to characterize their function during embryogenesis. Extracts from wild-type and dfmr1– (see Materials and methods) embryos were subjected to sucrose gradient velocity centrifugation in parallel (Fig. 1A). dFMRP sedimented at the top of the sucrose gradient in wild-type extracts, away from ribosomal subunits and active polyribosomes, and was absent from dfmr1– extracts (Fig. 1A). The top fractions from each gradient were pooled and subjected to specific and control immunoprecipitations (Fig. 1A). Proteins within each immunoprecipitate were then identified by multidimensional protein identification technology (MudPIT). Proteins immunoprecipitated with anti-dFMRP antibody from wild-type extract, but not found in either control immunoprecipitate (non-specific antibody and wild-type extract, or anti-dFMRP antibody and dfmr1– protein extract) were considered specific dFMRP-associated proteins. These stringent criteria identified only two proteins: eukaryotic initiation factor (eIF) 4G and the previously uncharacterized Drosophila homolog of the vertebrate cell cycle-associated protein (Caprin). eIF4G mediates the binding of all translationally competent mRNAs to the 40S ribosome to form a pre-initiation complex and is a major target of translational regulation (Pestova et al., 2007). Vertebrate Caprins are transcript-specific RNA-binding proteins implicated in translational regulation (Shiina et al., 2005; Solomon et al., 2007). Drosophila Caprin (CAPR) and human Caprins share a highly conserved Homology Region 1 (HR1) (32% identical/52% similar to human CAPRIN1), G3BP/Rasputin-binding domain (7/7 consensus core residues) and three RGG RNA-binding domains (Fig. 1B). The HR1 domain is the most highly conserved domain among the Caprin family members and Capr (CG18811) is the only HR1-containing gene in the Drosophila genome (Grill et al., 2004). The identification of only two dFMRP-interacting proteins, both translational regulators, validates the stringency of our screen and suggests that the proteins identified are relevant to the mechanism of dFMRP function.

Vertebrate Caprin1 has been shown to localize to neuronal granules within dendrites and to repress the translation of specific mRNAs implicated in synaptic plasticity (Shiina et al., 2005; Solomon et al., 2007). Because Caprin and FMRP had individually been implicated in translational regulation and synaptic plasticity, we set out to characterize the functional significance of the CAPR-dFMRP interaction. Immune sera raised against a CAPR C-terminal peptide specifically recognized a single 140 kDa band in wild-type adult or embryo extracts (see Fig. S1 in the supplementary material; Fig. 2B). A significant proportion of dFMRP and CAPR, and a relatively
small proportion of eIF4G, co-immunoprecipitated with both dFMRP and CAPR (Fig. 1C). Treatment of the immunoprecipitates with RNase A resulted in dissociation of the dFMRP-CAPR interaction but not that of eIF4G (Fig. 1D), indicating that dFMRP and CAPR co-immunoprecipitate through binding in a common ribonucleoprotein (RNP) complex and not through direct protein-protein interactions. Our immunofluorescence analysis of fixed wild-type cleavage stage embryos revealed that CAPR is cytoplasmic and appears to be enriched in previously described dFMRP-containing cytoplasmic RNP bodies (Monzo et al., 2006) (Fig. 1E). In gastrula stage embryos, CAPR and dFMRP are highly expressed in the central nervous system (see Fig. S2 in the supplementary material), consistent with the possibility that CAPR functions together with dFMRP in neurons as well as in the embryo.

dFMRP and Caprin collaborate to control timing of the MBT

To determine whether CAPR is required for embryogenesis, we generated mutations in Capr by imprecise P element transposon excision. Four ‘protein null’ alleles were recovered (Fig. 2A, B), and the largest deletion that affected only the Capr transcript (Capr2) was characterized for phenotypes. Capr2/Df(3L)Cat flies were viable and showed no obvious morphological defects. Time-lapse DIC microscopy of embryos derived from these females (hereafter referred to as Capr− embryos) showed that they developed normally until the MBT (NC14); cellularization, however, then occurred at a significantly reduced rate (Fig. 2C). This appears to be identical to the previously reported phenotype observed in the majority of dfmr1− embryos (Monzo et al., 2006).

To test whether dFMRP and CAPR functionally interact, we examined the phenotype of Capr2, +/Df(3L)Cat, dfmr13 mutant flies (hereafter referred to as Capr−, fmr1− embryos), which lack both copies of Capr and one copy of dfmr1. These mutants are viable but embryos laid by Capr−, fmr1− females display a novel phenotype, as revealed by time-lapse DIC microscopy, that is not observed in embryos from females lacking dfmr1 or Capr alone. Remarkably, 50% of cleavage stage Capr−, fmr1− embryos displayed a dramatic disruption in MBT timing. They initiated cleavage furrow formation normally but, instead of undergoing a prolonged interphase, they entered mitosis 14 prematurely. A similar phenotype is observed in haploid embryos, which do not achieve the species-specific N:C ratio required to trigger the MBT until NC15 (Edgar et al., 1986). During the premature mitosis the nascent cleavage furrows regressed, only to reform during interphase of NC15, when the embryos attempted to complete cellularization (Fig. 2C; Fig. 3; compare Movies 1 and 2 in the supplementary material). In some embryos this occurred uniformly, whereas in others it occurred in large patches (Fig. 4I,J,L; see Movie 2 in the supplementary material). In time-lapse DIC microscopy recordings of wild-type, Capr− and Capr−, fmr1− embryos, quantification of NC10–14 lengths showed no significant difference in nuclear cycle duration between wild-type and mutant embryos until NC14 (Fig. 3). Despite the dramatic disruption in the timing of the MBT, our analysis of live and fixed Capr−, fmr1− embryos revealed no signs of aberrant mitosis (Fig. 4). This is a significant departure from what has been observed in mutants of some other maternal-effect genes implicated in timing the Drosophila MBT (e.g. grp, mei-41 and smg), which display widespread mitotic spindle defects by NC13 (Fogarty et al., 1997; Sibon et al., 1997; Dahanukar et al., 1999; Sibon et al., 1999; Yu et al., 2000). Spindle morphology and nuclear spacing in Capr−, fmr1− embryos appeared normal during mitosis 13 (Fig. 4, compare A, B and C with F, G and H, and compare A', B' and C' with F', G' and H') as well as in those Capr−, fmr1− embryos undergoing premature mitosis 14 (Fig. 4I,J,L,L'.Lii). Therefore, although Capr function, like that of dfmr1, is essential for efficient cellularization, the combined reduction of Capr and dfmr1 function produces a distinct, earlier phenotype resulting from disruption in timing of the two morphological aspects of the MBT: prolonged interphase and cellularization.

Fig. 2. Maternal expression of Capr is required for the MBT. (A) Structure of Drosophila Capr showing start (ATG) and stop (TGA) codons, introns (thin lines), exons (gray boxes) and untranslated regions (light gray boxes). The P element transposon (EY06062) was excised to generate four deletion alleles. Black bars (1–4) indicate the deleted regions, none of which removes the 3′ end of the upstream gene (TAG stop codon). (B) Immunoblots with equal amounts of adult extracts were probed for Capr or Tubulin. +, wild type; Df, Df(3L)Cat (which removes Capr); EY, transposon insertion EY06062; 1–4, Capr alleles. (C) Frames from representative DIC movies of single embryos from females of the genotypes indicated. Capr− is a perfect excision of EY06062, with no detectable phenotype. Frames show a sagittal portion of each embryo at times (in minutes) relative to NC14 onset; the number of embryos displaying the phenotype over the total period analyzed is indicated (n). Arrowheads indicate the furrow front. The asterisk indicates premature mitosis 14 with furrow disassembly.

RESEARCH ARTICLE

DEVELOPMENT
Loss of dFMRP and Caprin function specifically alters Cyclin B and Frühstart protein levels at the MBT

Intriguingly, vertebrate Caprin1 was originally identified as a mitotic phosphoprotein (Stukenberg et al., 1997) and is highly expressed in proliferating tissues (Grill et al., 2004; Shiina et al., 2005), including activated lymphocytes, in which it is required for normal cell cycle progression (Wang et al., 2005). To determine whether any of the cell cycle control proteins might be targets of dFMRP/CAPR-dependent regulation, we assessed protein expression. Lysates of stage-matched wild-type, Capr– and Capr+/Df, fmr1– embryos were probed for proteins known to control the cell cycle during the MBT: CDC2, Cyclins A, B and B3, CDC20 (FZY) and Frühstart (FRS). Five developmental stages that span the MBT were analyzed for expression of candidate proteins (a single blot was probed for Fig. 5A; see Fig. S3 in the supplementary material). We looked for altered levels during the MBT in Capr–, fmr1– mutants relative to two controls (wild-type and Capr+ embryos) that never undergo premature mitosis 14. In Capr–, fmr1– mutants the steady-state levels of CYCB were significantly elevated during mitosis 13 and levels of CYCA also appeared to be somewhat elevated (Fig. 5A). In addition to prematurely elevated CYCB levels, Capr–, fmr1– embryos displayed delayed accumulation of the known, zygotically transcribed CDK inhibitor, FRS (Grosshans et al., 2003; Gawlinski et al., 2007) (Fig. 5A). The other proteins examined were unaffected.

The phosphorylation state of CDK1 (CDC2) at specific residues is known to contribute to the activation and inhibition of Cyclin-CDK1 activity (Nurse, 1990; Edgar et al., 1994). Although antibodies are not available to the phosphatases String/CDC25 and Twine/CDC25, the normal stage-specific CDK1 phosphorylation profile in all three genotypes during the MBT suggested that the balance of phosphatase and kinase activities that regulates CDK1 was unaltered. mRNA levels were also unaffected: changes in CYCB and FRS accumulation occurred despite normal steady-state levels of CycB mRNA and normal zygotic transcriptional activation of frs (see Fig. S4 in the supplementary material). Together, these data suggest that CAPR and dFMRP act to promote the shift in cell cycle at the MBT through suppression of CycB expression and activation of frs expression.

dFMRP and Caprin specifically associate in vivo with mRNAs encoding Cyclin B and Frühstart

To determine whether CycB and frs are themselves direct targets of dFMRP/CAPR translational regulation we tested whether the mRNAs specifically associate with CAPR and/or dFMRP at this time in development. CAPR and dFMRP immunoprecipitations were conducted from extracts of wild-type embryos, and mock immunoprecipitations were conducted from extracts of stage-matched protein null mutant embryos. Levels of specific mRNAs in the starting extract (steady-state levels) and in the immunoprecipitates were determined by quantitative RT-PCR. Both CycB and frs mRNAs were enriched in immunoprecipitations performed from wild-type extracts (Fig. 5B) suggesting that they are part of a dFMRP- and CAPR-containing mRNA-protein complex in vivo and are likely to be direct targets of translational regulation.

Elevation of CYCB levels in Capr–, fmr1– embryos contributes to disrupted timing of the MBT

If the elevated CYCB expression specifically observed in Capr–, fmr1– mutants is relevant to the MBT phenotype, then reducing the expression of maternal CycB would be predicted to suppress the phenotype. The premature mitosis 14 phenotype was typically observed in 50% of Capr–, fmr1– embryos. Reduction of maternal CycB by one half in this background produced partial rescue, such that only 17% of embryos underwent premature mitosis (Fig. 6), indicating that protein derived from maternal CycB contributes to the phenotype. Normally, expression of zygotic CycB is believed to occur only after the completion of cellularization (during gastrulation) (Dalby and Glover, 1993). To determine whether premature expression of zygotic CycB also contributes to the premature mitosis 14 phenotype we used a GFP-marked balancer to follow the zygotic genotypes of live embryos. Capr–, fmr1– females were crossed to CycB2/Cyo-GFP males and their embryos were imaged by DIC and fluorescence microscopy. Reduction of the zygotic contribution of CycB had no effect on the premature mitosis phenotype (Fig. 6). Together, these results suggest that the elevated level of CYCB observed during mitosis 13 is derived exclusively from the premature translation of maternal CycB mRNA in Capr–, fmr1– mutants and contributes significantly to the observed MBT phenotype.

---

Fig. 3. Reduction of CAPR and dFMRP function specifically disrupts timing of the MBT. The length of interphase (black line) for nuclear cycles (NC) 10–14 for Drosophila embryos derived from females of the indicated genotypes. Elapsed times (minutes ± s.d.) and number of embryos analyzed (n) are indicated beneath the line that represents each genotype. Note the precocious mitosis of embryos from Capr–, fmr1– females at 18.9 minutes into interphase of NC14. Minor differences noted in NC12 and NC13 interphase and mitosis lengths produced no net change in overall cycle length.
DISCUSSION

In summary, we propose that maternal dFMRP and CAPR associate to regulate translation of specific mRNAs and that this regulation is essential to the embryo at a critical and sensitive juncture – the MBT. Specifically, following normal degradation of cyclins during NC13 metaphase/anaphase, Capr–, fmr1– embryos synthesize CYCB prematurely from maternal mRNA and delay synthesis of FRS from zygotic mRNA. The resultant imbalance at this particular time disrupts the cell cycle. FRS expression normally first occurs at the MBT and, not surprisingly, premature ectopic expression can block earlier mitoses (Grosshans et al., 2003). The exquisite sensitivity of M-CDK1 activity to levels of CYCB at the MBT is consistent with evidence that exogenous CYCB protein can induce premature mitosis within the first 5 minutes of cellularization (NC14 interphase), whereas similar elevation of CYCB levels 15 minutes into cellularization cannot (Royou et al., 2008). It is also possible that the stochastic patches of precocious mitosis that we observe in our mutants result from small local differences in the level of CAPR- and dFMRP-containing mRNPs formed, as the responding machinery is particularly sensitive to levels of CYCB at this time. Levels of specific mRNAs are unaffected in our mutants at the initiation of NC14, so altered CYCB and FRS levels could arise in principle through altered protein synthesis or protein stability. However, it is unlikely that the elevated CYCB we observe reflects faulty degradation because chromosome segregation and mitotic exit were unaffected (Fig. 3, Fig. 4). Furthermore, all cyclins examined depend on anaphase-promoting complex/cyclosome (APC/C)-FZY/CDC20 for their degradation (Sigrist et al., 1995; Raff et al., 2002; Huang et al., 2007), and both FZY expression and APC/C activity, as evidenced by appropriate CYCB3 degradation, appeared to be normal (Fig. 5A). Therefore, we believe precise translational regulation by dFMRP and CAPR is necessary to successfully negotiate the MBT.

In order to understand how translational regulators can so accurately modulate complex and diverse programs, it will be necessary to determine the mechanism by which proteins such as dFMRP and CAPR are recruited to specific target mRNAs and modulate translation. Activation or repression could arise from distinct regulatory mRNPs assembled on each specific mRNA region (reviewed by Darnell et al., 2005a). For example, RNA structure could induce conformational changes as a protein binds, or vice versa. Several motifs have been reported to mediate RNA binding by FMRP in vitro or in vivo: G-quartets (Darnell et al., 2001), kissing complex (Darnell et al., 2005b) and SoSlip (Bechara et al., 2009). Our analysis using RNABOB (http://selab.janelia.org/software.html), BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) or Mfold (http://mfold.bioinfo.rpi.edu/cgi-bin/rna-form1.cgi) did not identify any of these in the frs and CycB mRNAs, suggesting the association of these mRNAs with dFMRP and CAPR might be mediated by novel sequence motifs or might rely on the binding of additional proteins or on RNA structures not identified by these algorithms. Once assembled, dFMRP- and CAPR-containing mRNPs might modulate translation rates directly or by affecting the localization or stability of specific mRNAs. It is intriguing in this regard that the absence of CAPR leads to partial stabilization of CycB mRNA subsequent to the observed precocious mitosis phenotype, and the stabilization is greater upon reduction of dFMRP (see Fig. S4 in the supplementary material). The binding
A role for dFMRP and Caprin at the MBT

Fig. 5. dFMRP and CAPR associate with CycB and frs mRNAs in wild-type embryos and specifically alter CycB and FRs protein expression at the MBT. (A) An immunoblot of ten Drosophila embryos per lane of the indicated genotypes (top) was probed for the indicated proteins (left), which included Lamin (LAM) as a loading control. Hand-sorted embryos were of the indicated stages: NC13 interphase (I13) and mitosis (M13), or NC14 early, middle and late interphase (I14). Phospho-isoforms of CDC2 (Edgar et al., 1994) are indicated proteins (left), which included Lamin (LAM) as a loading control. Relative levels of CycB and FRs were determined by quantitative PCR. Levels are presented as ratios (wild type/mutant) of normalized mRNA values using a two-tailed Student’s t-test. *P=0.022 for CycB and P=0.020 for frs; **P=0.003 for CycB and P=0.00005 for frs.

Fig. 6. Elevated levels of maternally derived CycB promote premature mitosis at the MBT. The percentage of embryos (n, number of embryos analyzed) derived from the indicated crosses (beneath) that display a premature mitosis 14 phenotype. Progeny derived from Capr2+/Df, dfmr113 females receiving either a CycB2 (light-gray bar, GFP+) or GFP-marked (dark-gray bar, GFP+) paternal chromosome displayed the premature mitosis 14 phenotype at similar frequencies. The reduction of maternal CycB mRNA partially rescues the precocious mitosis 14 phenotype (left panel, far right-hand bar), whereas the reduction of zygotic CycB mRNA does not (right panel, GFP).

of CAPR and dFMRP might therefore also be required to ensure appropriate degradation of CycB mRNA after the onset of the MBT.

How might dFMRP and CAPR directly modulate translation? In the case of FMRP there is general agreement that it is a transcript-specific translational regulator; however, whether it functions as a repressor or activator, and whether it modulates initiation, elongation and/or termination, remain controversial. The overall distribution of polysomes between wild-type and dfmr113 extracts was indistinguishable under our conditions, suggesting no gross change in the levels of translation (Fig. 1A). Since the majority of dFMRP does not co-sediment with active polysomes but can associate with eIF4G, a key scaffold for pre-initiation complex assembly (Fig. 1C, D), our data suggest that dFMRP in early embryos regulates the translational initiation of specific transcripts. Caprin has also been implicated in the control of local translation required for synaptic plasticity (Richter and Klann, 2009; Wang et al., 2010), however, as with FMRP, the mechanism remains unresolved. The presence of CAPR in cap-binding complexes from Drosophila ovariates (Pisa et al., 2009) suggests that Caprin, like dFMRP, might regulate initiation complex assembly through interactions with eIF4G, consistent with the RNA-independent association of eIF4G in our CAPR immunoprecipitates. Current data from vertebrate studies indicate mitotic phosphorylation and binding to G3BP, a Ras effector, as two likely modes for regulating CAPR function in response to developmental signals. In Drosophila, conservation of the G3BP-binding domain of CAPR, the high levels of expression of the G3BP ortholog Raspitun in early embryos (Pazman et al., 2000), and the presence of Raspitun in cap-binding complexes (Pisa et al., 2009), suggest that these regulatory mechanisms are likely to be conserved. Biochemical studies will be required to assess how eIF4G associates with dFMRP- and CAPR-containing complexes, and whether dFMRP or CAPR indeed affects the assembly of functional pre-initiation complexes or modulates translation through a distinct mechanism(s).

Although CycB and frs are targets of translational control during the MBT, it is likely that dFMRP and CAPR regulate additional targets, together or individually, to help ensure reliable completion of all aspects of this developmental transition and potentially other similarly dynamic transitions. Currently, FMRP is believed to regulate hundreds of mRNAs in neuronal dendrites (O'Donnell and Warren, 2002), and although CAPR is reported to function similarly, their interaction in this tissue has yet to be addressed. Similarly, CAPR has been implicated in the activation of lymphocytes (Grill et al., 2004; Wang et al., 2005), a role yet to be investigated for dFMRP. Intriguingly CAPR, dFMRP and Raspitun co-immunoprecipitate from Drosophila ovary extracts (Costa et al., 2005), suggesting that the germ line might be another tissue that requires their combined action. In addition to roles in development, FMRP and CAPR are implicated in rapid translational responses to stress. When tissues are exposed to particular forms of stress they respond with the formation of stress granules, which are RNA-sorting and -processing foci that contain 40S ribosomal subunits along with FMRP, CAPR and G3BP (reviewed by Anderson and Kedersha, 2009; Buchan and Parker, 2009). Our data indicate that FMRP and CAPR cooperate to modulate the timing of the MBT in...
early embryos and lead us to postulate that the ability to respond to developmental signals rapidly through transcript-specific translational regulation might underlie a number of developmentally significant transitions.

Acknowledgements

We thank J. Sierra (Universidad Autonoma de Madrid), J. Groshans (ZMBH, Heidelberg), J. Wilhelm (U.C. San Diego), T. Kaufman (Indiana University), J. Raff (The Gurdon Institute, Cambridge, UK), P. Fisher (SUNY, Stonybrook) and C. Lehner (University of Zurich) for the generous gift of reagents, and D. Bilder (U.C. Berkeley) and the Patterson 2nd Floor Writing Workshop participants for helpful comments on the manuscript. This work was funded by NIH P41 RR011823 grant support to J.R.Y. and NIH R01 GM097562 to J.C.S. Deposited in PMC for release after 12 months.

Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.055046/-/DC1

References


<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>RpL32</td>
<td>GCGCACCAAGCACCTTCATC</td>
</tr>
<tr>
<td>RpL32</td>
<td>GACGCACTCTTGTGATACC</td>
</tr>
<tr>
<td>cdc2</td>
<td>AGGGTGCAACCCGCTGAC</td>
</tr>
<tr>
<td>cdc2</td>
<td>CTCCATCAAACATCTCCAACAG</td>
</tr>
<tr>
<td>CycB</td>
<td>CCGAGGACAGACACCATAACG</td>
</tr>
<tr>
<td>CycB</td>
<td>CTGTGTGCTGGAGATGGTCCTTC</td>
</tr>
<tr>
<td>frs</td>
<td>TCCCTGGACAGAAAGATTTCC</td>
</tr>
<tr>
<td>frs</td>
<td>CGCTTTTTGATCGGTGTTTG</td>
</tr>
</tbody>
</table>